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Valorization of the Seeds (Almonds and Oil) of the Spontaneous Argan of Tindouf and the Other Experimental Domesticated Argan of Mostaganem in Algeria

Benaouf Zohra, Djorf Oussama, Jaradat Chawkat and Kechairi Reda

Abstract

The aim of the research was to determine the phytochemical and parameters of argan oil and almonds. We are interested in following the formation of volatile compounds in argan oil and also the determination of antioxidants; the purpose was mainly to identify and quantify the antioxidants to meet this objective, two samples of argan oil from the almonds of Tindouf taxa and Mostaganem taxa. The results show that the argan rich in phenolic compounds deserve to be exploited as much as nutritional and pharmaceutical supplements because of their antioxidant properties, which can surely contribute to the safeguarding of the argan tree.

Keywords: argan oil, almonds, volatile compounds, parameters, phenolics, antioxidant

1. Introduction

The argan tree, being a xerophile species, observed on the semiarid and arid climate has specific ecological characteristics and many interests (forest, forage, and fruit). Argan oil is essentially rich in unsaturated fatty acids and saturated fatty acids. As for the secondary metabolism, it contains polyphenols, tocopherol, sterol, and alcohol, and this explains its benefits in treating heart diseases and skin infections and in general its therapeutic uses and medication as a food supplement [1, 2]. Argan oil has a high level of oleic and linoleic acids and antioxidant compounds, which has an impact on cardiovascular disease [3]. Minor compounds of argan oil, such as sterols, may be involved in its cholesterol-lowering effect [4]. The antidiabetic effect of argan oil has been claimed for a long time in traditional medicine; however the mechanism of regulation of the level of glucose in the blood remains unknown [5]. The antihypertensive effect of argan oil and its mechanism of action have been studied by Berrougui et al. [6]. The purpose of this present work is the

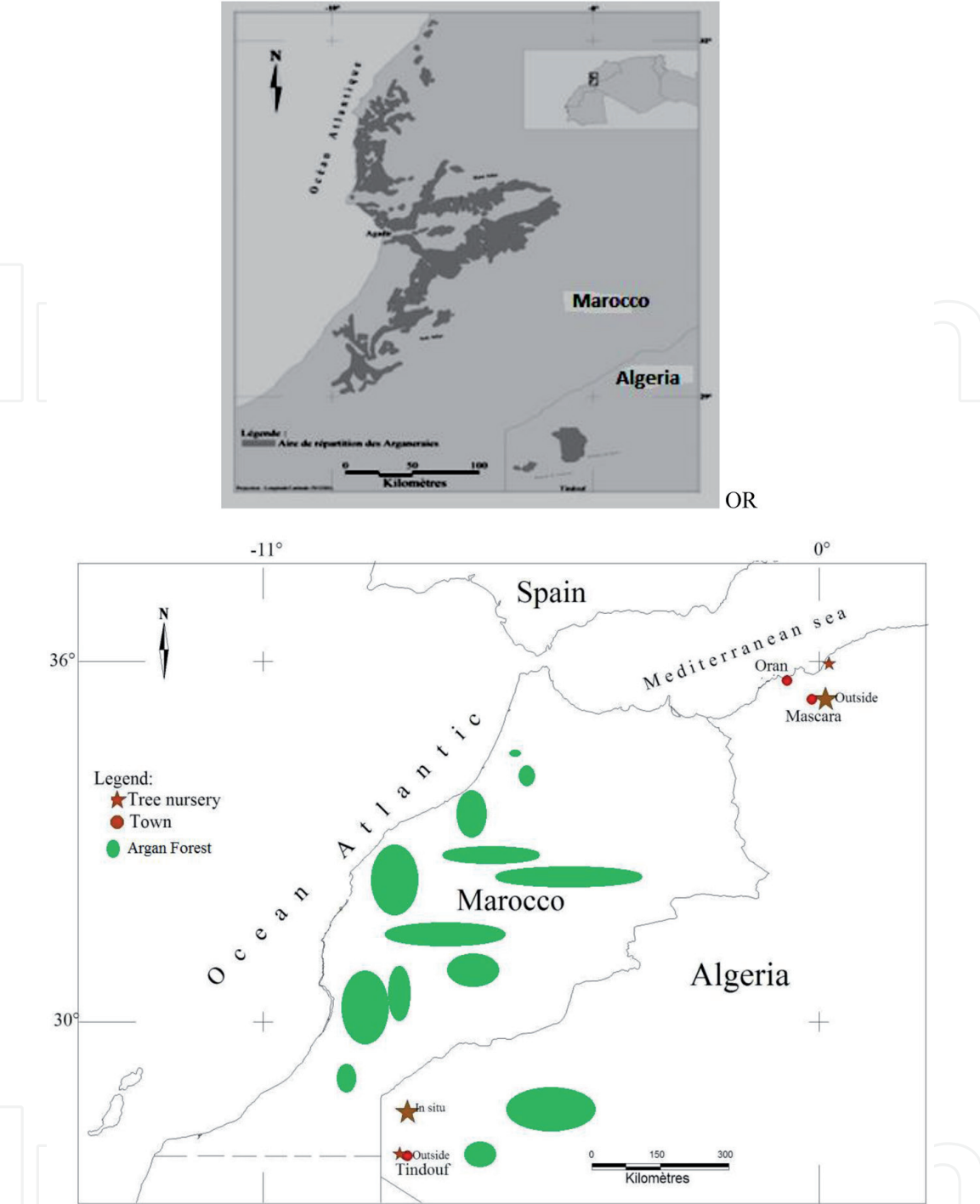


Figure 1.
Distribution of the argan tree in Tindouf and northwest Africa [7].

comparison between two provenances of argan tree, an endemic variety that grows in southwestern Tindouf located in Algerian Sahara and the other introduced to Mostaganem located in Mediterranean area (**Figure 1**).

2. Materials and methods

2.1 Plant material

A mature fruit of Algerian argan (*Argania spinosa*) was collected from Tindouf area located in Tindouf and Mostaganem (coastal region) in June 2016; almonds and the extracted oil were analyzed.

2.2 Argan oil preparation

The extraction was carried out by a Soxhlet apparatus, according to the standard technique [8]; the technique consists in using an organic solvent (hexane). 25 g of almond seeds powder are placed in a cartridge, and then the cartridge is closed by cotton and placed in the Soxhlet extractor. A flask is weighed empty and then filled with 200 ml of solvent. This flask is inserted into the extractor and placed in a sand bath set at a boiling point of the solvent. The extraction is carried out for 3 h and 6 h, then the solvent is removed by distillation, and the oil which remains in the flask is dried at a temperature of 105°C for a few minutes. The volatile compounds were extracted by the solid-phase microextraction (SPME) method; this technique does not require the use of solvents or complicated apparatus, and it is based essentially on the adsorption phenomenon based on a balance between the matrix and coating of the fiber. The identification and quantification of aromatic compounds were performed by gas chromatography-mass spectrometry (GC-MS) (**Figure 2**).

The chemical parameters were detected according to ISO standards [9, 10]. Total sugars were measured according to the method of Dubois et al. [11].

Equipment related to the results presented in **Table 1**: 50 mg of the almonds of each sample and put in a vial, distilled water is added until at 50 ml. Introduce 1 ml of the solution to be assayed into a tube of each sample and then 1 ml of the phenol solution (5%). The tubes are carefully shaken, and then 5 ml of concentrated

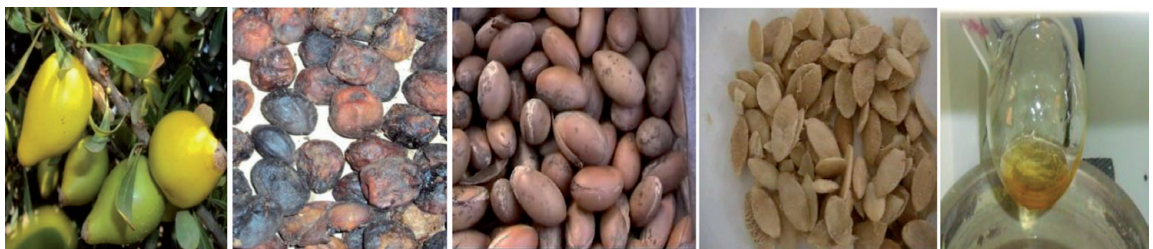


Figure 2.
Fruit, seed, and almond argan oil.

Parameters	Tindouf argan	Mostaganem argan
Relative density	0.83 ± 0.02	0.91 ± 0.03
Refractive index	1.4642 ± 0.08	1.4612 ± 0.04
Acid number	2.244 ± 0.01	2.524 ± 0.09
Index saponification	179.55 ± 0.8	185.60 ± 0.5
Ester index	177.306 ± 0.3	183.076 ± 0.5
pH	4.62 ± 0.07	4.43 ± 0.02
Humidity	2 ± 0.001%	4.33 ± 0.002%
Phosphatide	11.4 ± 0.06%	13.8 ± 0.02%
Extraction yield (3 h)	25.727 ± 0.08%	25.727 ± 0.02%
Extraction yield (6 h)	25.727 ± 0.07%	41.67 ± 0.04%
Total sugar	8.19 ± 0.04%	4.86 ± 0.06%
Fat	38.61 ± 0.3%	41.67 ± 0.5%
Ash	2.4 ± 0.03%	1.4 ± 0.01%
Nitrogen content	1.045 ± 0.001%	0.602 ± 0.00%
Protein	6.53 ± 0.04%	3.76 ± 0.005%

Table 1.
Chemical and physical parameters of argan oil of two taxa, Tindouf and Mostaganem argan.

sulfuric acid " H_2SO_4 " are added using a graduated pipette. After standing for 30 min in the dark, the absorbance (OD) measurements are made at 490 nm in the case of hexoses. The calibration of the spectrophotometer (UV-vis spectrophotometer) is done with a blank solution containing 1 ml of distilled water, 1 ml of 5% phenol, and 5 ml of H_2SO_4 .

The mineral material was determined by 5 g of the almonds of each region placed in the capsules and placed in the muffle furnace with a temperature of 900°C for 2 h and then metered in desiccators until it was cooled and finally weighed. The protein content is carried out in three stages: mineralization, distillation, and titration; in each flask 3 g of sample from each region are introduced, and 1.5 g of the catalyst is added with some glass bead, then 20 ml of sulfuric acid are poured in. A concentrated 50 ml of distilled water and 45 ml of sodium hydroxide solution (40%) are added for 3 min. The end of the apparatus is leveled in a tarpaulin containing 20 ml of boric acid (4%) which fixes the solution. The titration is carried out with a 0.1 N sulfuric acid solution in the presence of a colored indicator (methyl red) until a pink turn is obtained. For the determination of the fat, introduce 50 g of the sample from each region into the cartridge, place it in the Soxhlet, and weigh the empty flask and fill it with hexane (300 ml). After 6 h of extraction, determine the moisture by the loss of sample water (oil), take two capsules, put in each capsule 3 g of the oil, and placed in an oven at a temperature of 105°C for 2 h of drying. For the determination of the refractive index, calibrate the refractometer apparatus with distilled water. Then, one or two drops of each oil sample are placed on the prism, and the dark zone is moved in the middle for the separation cloth of the light and dark beach.

Determination of PH and acid number, 2 g of the sample and introduce it into the flask or flask. Add 5 ml of ethanol and some drops of phenolphthalein solution (or phenol red) as an indicator, and titrate the liquid with the potassium hydroxide solution contained in the burette to the color curve where the volume V is recorded. For the determination of saponification index, in a flask introduce 2 g of the sample, and add with a burette 25 ml of potassium hydroxide solution and fragments of pumice or porcelain. Fit the glass tube or refrigerant, and place the balloon on the boiling water bath. Allow to cool, disassemble the tube, and add 20 ml of water then 5 drops of phenolphthalein solution. The ester number is the number of milligrams of potassium hydroxide necessary for the neutralization of the acids released by the hydrolysis of the esters contained in 1 g of argan oil. Hydrolysis of the esters by heating in the presence of an ethanoic solution, determination of the excess of alkali by a standard solution of hydrochloric acid. For the determination of phosphatide content, introduce 25 g of oil and 200 ml of acetone in a flask, then leave the mixture at a temperature of 4°C for 2 hours, then filter the mixture on the paper previously weighed and dry this paper at a temperature of 100°C up to 150°C , and finally put in the desiccator and weigh.

2.2.1 SPME sampling conditions

Analysis was performed as described by Baccouri et al. [12]. Each oil sample was spiked with 4-methyl-2-pentanone (internal standard) to a final concentration of $6.7\text{ }\mu\text{g/kg}$. Then 1.5 g was introduced into a 10 ml vial fitted with a silicone septum. The vial was immersed in a water bath at 40°C , and the oily solution is maintained under magnetic stirring. After 2 min, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm , 2 cm long from Supelco Ltd., Bellefonte, PA) was exposed to the sample headspace for 30 min [13] and immediately desorbed for 2 min at 260°C in the gas chromatograph in splitless condition. All the analyses were performed in triplicate.

2.2.2 GC-MS analysis

GC-MS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 mm film thickness, J&W Scientific Inc., Folsom, CA, USA). Due to the high boiling point of the oily compounds, direct injection to GC-MS apparatus is impossible, and pre-preparation has to be done. We used increased temperatures. Detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode, using ionization energy of 70 eV. The identification of volatile compounds was confirmed by the injection of pure standards. Compounds for which pure standards were not available were identified on the basis of mass spectra and retention indices available in the literature. The relative concentration ($\mu\text{g kg}^{-1}$ of oil) of the identified compounds was calculated by relating the areas of the internal standard of each compound.

2.3 Spectrophotometric assays

2.3.1 Determination of total phenolic content (TPC)

The amount of total phenolics was assayed spectrophotometrically by means of the modified Folin-Ciocalteu method [14, 15]. Briefly, 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent, 2 ml of 7.5% aqueous sodium carbonate solution, and 0.5 ml of phenolic extract were mixed well. After 15 min of heating at 45°C, the absorbance was measured at 765 nm with a UV-visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Milan, Italy) [16].

2.3.2 Determination of DPPH radical scavenging activity (RSA)

The hydrogen-donating ability of the crude extract and radical scavenging activity (RSA) of argan fruit parts were investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging assay (RSA) [17, 18]. All operations were done in the dark or dim light [19]. For control purpose, the absorbance of the DPPH• without samples was measured.

The inhibition percentage (IP) of the DPPH• by the extracts was calculated according the formula $IP = [(A_{0\text{min}} - A_{60\text{min}})/A_{0\text{min}}] \times 100$. With the percentage of remaining DPPH• being proportional to the antioxidant concentration in the extracts, the DPPH• scavenging activity was expressed as μM of Trolox equivalent (TE) per mg of sample.

2.3.3 Determination of total antioxidant activity (TAA)

The TAA in crude extracts was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay following the original analytical procedure described by Re et al. [20] with slight modifications. ABTS radical cation ($\text{ABTS}^{\bullet+}$) was produced by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration). For the study, the $\text{ABTS}^{\bullet+}$ stock solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30°C. Sample solutions of 30 μL (or standard) were mixed with $\text{ABTS}^{\bullet+}$ solution 3 ml. Absorbance readings were taken at 30°C exactly 6 min after initial mixing. An appropriate solvent blank was obtained by mixing an absolute ethanol of 30 μL with $\text{ABTS}^{\bullet+}$ solution of 3 ml and monitored its absorbance at 6 min. All determinations were carried out in triplicate. The $\text{ABTS}^{\bullet+}$ scavenging

effect (% Inhibition) was calculated by the equation $\% \text{ Inhibition} = [(A734\text{blank} \times A734\text{sample}) / A734\text{blank}] \times 100$ where $A734\text{blank}$ and $A734\text{sample}$ are the absorbances of ABTS•+ solution at 734 nm before and after the sample addition. Calibration was performed, as described previously, with Trolox stock solutions. Results were expressed as μM Trolox equivalent (TE) per mg of sample.

2.4 Statistical analysis

Significant differences among different oils were tested by the one-way analysis of variance and the Duncan test for mean comparison. Statistical analyses were performed using the software package Statistica version 7. Results were reported as mean \pm standard deviation ($n = 3$). The analysis of variance (one-way ANOVA) was performed with SPSS software (version 12.0 for Windows, SPSS Inc., Chicago, Illinois). Duncan's test was applied to assess significant differences among the variables ($p < 0.05$), while Pearson correlation test was used to show their correlations.

3. Results and discussion

We noted a small difference in the extraction yield of almonds for both Tindouf and Mostaganem taxa, respectively (25.727–29.272%), after the 3 h duration, while they have a difference of 38.63–41.67% for the duration of 6 h. The percentages of the total sugar of Mostaganem taxa kernels (4.86%) are equal to almost half of the percentage of Tindouf taxa total sugars (8.19%). The sample of Tindouf taxa kernels contains a significant ash (2.4%) compared to the Mostaganem taxa sample (1.4%). The protein content of Tindouf almonds (6.35%) is high compared to the Mostaganem taxa (3.76%). As for the amount of fat, it is brought closer together between the two samples; the kernel gives a significant amount of (40%). Concerning the physicochemical characteristics of argan oil, according to our results we notice that argan oil is not miscible with ethanol for both samples. A difference in the moisture content between the argan oil of the Mostaganem taxa (4.33%) and the Tindouf taxa (2%), and the relative density, by comparison the Tindouf oil (0.83) is lower than Mostaganem (0.91). On the other hand, for the refractive index of argan oil, we record the same values 1.46) with an acid pH. Regarding the other indices, the acidity index of the oil of Mostaganem taxa is equal to 2.5245 and that of Tindouf is 2.2440; the saponification index of the oil of Mostaganem is 185.6 and that of Tindouf is 179.55 (**Table 2**).

The results show some volatile compounds, including compounds of lipid peroxidation, Strecker degradation, and Maillard reaction, responsible for the formation of pyrazines and autoxidation of fatty acids. This study could help to adjust the argan oil aroma and perhaps meet new types of consumers. For the phytochemical part, we have undertaken a study on the volatile composition of argan oil. Extraction of the volatile compounds was carried out by solid-phase microextraction (SPME), and their identification and quantification were performed by gas chromatography-mass spectrometry (GC-MS). Finally, we were interested in the elucidation and quantification of polyphenols. These secondary metabolites are of great importance because of their antioxidant properties. In total, 11 phenolic compounds were identified and quantified in the argan tree. This could be achieved through the coupling of liquid chromatography and electrospray negative ion mass spectrometry (LC-ESI-MS). Among the polyphenols cited are procyanidins B1 and B2, (+)-catechin, (–)-epigallocatechin gallate, (–)-epicatechin, isoquercitrin, hyperoside, rutin, phloridzin, myricetin, and quercitrin. The unroasted kernels and the shell are characterized by a diverse phenolic composition. The pulp is

Compound	Tindouf argan	Mostaganem argan	<i>p</i>
<i>Acids</i>			
Butanoic	1.2 ± 0.2d	1.5 ± 0.5d	**
Valeric	1.2 ± 0.3b	1.5 ± 0.2d	***
Hexanoic	5.2 ± 1.5b	4.9 ± 2.6b	**
<i>Alcohols</i>			
1-Butanol	1.6 ± 0.02	1.5 ± 0.43	ns
1-Pentanol	7.5 ± 0.4b	11.6 ± 2.4c	***
1-Hexanol	20.8 ± 1.5	20.5 ± 2.6	ns
2-Heptanol	0.7 ± 0.1a	1.1 ± 0.4a	***
2,3-Butanediol <i>d,l</i>	40.2 ± 4.3	34.3 ± 5.2	ns
<i>Aldehydes</i>			
Hexanal	26.1 ± 0.8b	28.9 ± 5.89	***
Benzaldehyde	1.2 ± 0.1bc	1.4 ± 0.9c	***
<i>Esters, lactones</i>			
Ethyl 2-methyl butanoate	0.1 ± 0.01ab	0.1 ± 0.03a	***
<i>i</i> -Amyl acetate	1.2 ± 0.03c	0.8 ± 0.4c	**
μ-Butyrolactone	2.2 ± 0.6c	3.6 ± 1.2c	**
<i>Ketones</i>			
2-Heptanone	5.2 ± 0.4b	4.8 ± 1.2c	***
Acetoin	5.4 ± 0.8ab	4.2 ± 1.4ab	***
2-Undecanone	4.2 ± 0.2ab	3.1 ± 0.6ab	***
<i>Terpene</i>			
Limonene	0.3 ± 0.4a	0.25 ± 0.6b	**
<i>N-heterocycle</i> μg/kg (it is not %)			
1-Methyl-1 <i>H</i> -pyrrol	158.4 ± 22.1a μg/kg	147.1 ± 15.8b	**
2-Methyl pyrazine	121.3 ± 34.0ab	138.2 ± 36.5c	***
2,6-Dimethyl pyrazine	263.4 ± 42bc	273.1 ± 40.8d	**
2,3-Dimethyl pyrazine	6.3 ± 0.1ab	7.5 ± 0.9c	**
2-Ethyl-5-methyl pyrazine	24.9 ± 1.5a	23.4 ± 1.6c	***
2-Ethyl-6-methyl pyrazine	35.3 ± 4.2b	37.5 ± 3.2c	***
Trimethyl pyrazine	26.4 ± 1.2bc	49.8 ± 3.2d	***
2-Ethyl-3,5-dimethyl pyrazine	26.9 ± 0.5c	33.2 ± 0.9d	***
<i>Furans</i>			
2-Pentyl furan	23.1 ± 0.5ab	22.5 ± 5.8b	*
Furfurol	72.3 ± 1.0a	85.4 ± 4.4b	***
2-Furanmethanol	12.6 ± 0.7b	14.5 ± 2c	***
<i>q</i> , quantifier ion. Different letters in the same row at mean concentration values indicate significant differences (<i>p</i> < 0.05) as analyzed by Duncan test. * <i>p</i> < 0.05. ** <i>p</i> < 0.01. *** <i>p</i> < 0.001.			

Table 2.
Quantified volatile compounds (μg/kg of oil ± SD) isolated in argan oil of two taxa.

quantitatively rich in total polyphenols (69.53 mg gallic acid equivalent/g). It showed a free radical scavenging activity, measured by DPPH. Important relative to other parts of the fruit ($0.12 \pm 0.004 \mu\text{M}$ Trolox equivalents/mg) and antioxidant activity (ABTS•+) ($0.287 \pm 0.05 \mu\text{M}$ equivalent/mg Trolox). Interestingly, the results obtained confirm that argan fruit polyphenols deserve to be exploited as much as nutritional and pharmaceutical supplements because of their antioxidant properties, which can surely contribute to the safeguarding of the argan tree. The aim of this work was to identify and quantify the phenolic compounds of argan fruit and by-products of argan oil extraction. Total phenolic content and antioxidant activity by DPPH and ABTS were evaluated. The LC-MS examination resulted in the detection of 10 compounds of which 8 were unambiguously identified. The identified compounds are classified into three groups: flavanols, flavonols, and dihydrochalcones. The results showed that six compounds were detected in the pulp: isoquercitrin and hyperoside are predominant (25.8 and 18.5 mg/100 g, respectively); they are followed by rutin (7.2 mg/100 g) and quercitrin (0.32 mg/100 g). Epicatechin and procyanidin B2 were also detected but could not be quantified. The phenolic compounds of the fruit shell of the argan tree have not been the subject of any prior work. The major phenolic compound isolated from the shell is (–)-epicatechin (0.45 mg/100 g), followed by isoquercitrin (0.32 mg/100 g). Rutin and phloridzin have the same level (0.18 mg/100 g), hyperoside and procyanidins B1 and B2 both 0.08 mg/100 g, myricetin 0.04 mg/100 g, and finally the quercitrin that was detected could not be quantified. As for kernels and meal, a major compound was detected; however this compound could not be identified by Tandem mass spectrometry (Mw = 423.5, Rt: 8.5 min).

4. Conclusion

The average oil density of Mostaganem taxa seems low compared to Tindouf oil. For the refractive index, a small difference is noted between the two taxa of *Argania spinosa* oil. For the acid index, the sample of the Mostaganem area seems to be richer in free fatty acids than in the Tindouf area. Same for the saponification index and the ester index, it is important in the argan oil of Mostaganem than Tindouf. For the phosphatide content, it seems high in both zones, but Mostaganem oil is richer. Also our results show that the phenolic fractions studied have remarkable antioxidant properties. Although the composition of the phenolic fraction of fruits can evolve over the years, they deserve a better valuation in the pharmacological, cosmetic, and agro-food fields because of their antioxidant properties.

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